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NUTRITION REVIEWS

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NUTRITION REVIEWS

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NUTRITIONAL CONSIDERATIONS IN DESIGNING COMBAT RATIONS

To be able to conduct military operations in any area of the world has come to be an absolute requirement for our own and other armed forces. Modern transport facilities can surmount all geographic barriers, but the efficiency of our armed forces is dependent not upon machines but upon the ability of individual men to operate under extremes of climate and terrain. Fed poorly, their fighting efficiency drops. The objective of rations development is, therefore, to provide the food that will assure fighting efficiency under all circumstances.

Tactical situations, climate, terrain, and feeding methods and equipment create a large number of feeding problems which are met by a number of different rations, supplementary packs and food packets.

Rations must be evaluated in terms of military needs. Unless these rations, supplements and packets possess the functional qualities which are necessary or desirable under the circumstances of their use, they will fail wholly or in part to fulfill their purpose in the field. The success of a ration in meeting this challenging objective is measured in terms of four military characteristics—these are the guide lines of ration planning—(a) nutritional adequacy—should possess maximum nourishment compatible with the tactical situation and have no detrimental physiologic effects (*e.g.*, abnormal thirst, gastric disturbance); (b) acceptability—should provide sufficient variety to be palatable and acceptable when consumed *hot* or *cold* for several consecutive days; (c) stability—should be stable for two years at fluctuating temperatures ranging from −80°F. to 160°F. and relative humidity from 10 to 90 per cent; (d) utility—should be economical of weight and space, suitable for the individual to carry, easily prepared for consumption under all climatic conditions, and capable of withstanding military handling during transportation, storage and issue.

With the arrival on the military scene of new weapons—rockets, guided missiles, jet propelled planes, long range bombers, snorkle-equipped submarines—the complexity of armed forces feeding problems has vastly increased. If these weapons are to provide an advantage over the enemy, the men who operate them must also have a superiority in physical fitness that can be achieved by adequate nutrition. One stubborn obstacle stands in the path of an easy attainment of nutritional adequacy, namely, the severe limitation imposed by the essential requirements of acceptability, stability, and field utility.

To determine quantitatively whether or not a ration is nutritionally adequate it is necessary to compute the total nutritive value from reliable data on the nutrient content of component ration items. Conventional tables of food composition are not a valid source of nutrient data for most components of packaged rations. Almost all of the published data represent naturally occurring or standard foods in general use, whereas a majority of the ration components are specially formulated to meet military requirements. It is necessary, therefore, to determine by laboratory analysis the nutrient content of ration items manufactured according to formulations prescribed by specifications. Furthermore, the effects of storage are such that the average values given in standard tables of food composition may not apply to ration items. To integrate properly all aspects of nutritive values of

rations, a centralized source of this type of information must be established and kept up-to-date.

The food composition reference manual just completed is based on extensive data obtained from Quartermaster Food and Container Institute analyses. The manual lists the nutritive values of standard ration unit weights of all packaged ration components.

The Quartermaster coordinated research and development program on dehydration has drawn attention to the need for more information on the nutritive value of dehydrated fruits, vegetables, meats and eggs. Dehydrated commodity items have great utility advantages for both military and civilian feeding in time of emergency. Dehydrated white potatoes already have a wide-scale use in the standard B rations.

Although operational rations produce fairly good growth in rats, a greater growth rate is observed when the rations are supplemented with proteins and vitamins. Packaged rations which contain processed foods may be in actuality incomplete in nutrients despite the fact that they appear to meet, by calculation, nutritional standards. It is important, therefore, to devise and conduct feeding tests with laboratory animals (rats, guinea pigs, chicks, dogs and monkeys) to ascertain the physiologic availability of the nutrients in packaged rations. Such studies are useful as a guide to possible nutritional inadequacies.

Extremes of storage temperatures and long military supply lines explain the significant losses of nutritional value before rations reach the fighting men. The effects of storage under various known conditions of temperature and humidity on the nutrient stability and physiologic availability of rations as consumed must be determined. It is also desirable for practical reasons to establish a correlation between laboratory storage tests and military storage for loss of nutritional factors in rations.

Animal experimentation is useful as a "pilot test" for providing guiding information on the nutritional adequacy of operational rations. However, due to qualitative, but particularly quantitative, species differences between laboratory animals and man in their nutritive requirements, information secured from animals is frequently inapplicable to man. Under stress conditions the laboratory animal generally responds so differently, in kind or degree, from the human subject as to render comparison dubious. It is, therefore, essential to ascertain the significance of feeding tests with laboratory animals in relation to the nutritional welfare of members of the armed forces before proceeding to ration modifications. To achieve this purpose, arrangements have been made for collaboration with the Quartermaster Climatic Research Laboratory in using enlisted personnel for studying the relationship of nutrition to climatic stress. To impart realism and vitality to laboratory investigations of combat rations, field studies in collaboration with other national military establishment agencies will also be a profitable source of data.

It is a responsibility of the Surgeon General to prescribe basic standards of diet for the armed forces. Army Regulation 40-250 dictates the minimum allowances of nutrients which a ration must supply in order to be considered adequate. These values, patterned after the recommendations of the National Research Council, were modified in the light of previous experience to meet the needs of the Army. They now reflect what are considered to be practicably obtainable levels.

In normal civilian feeding and when troops are maintained on Field Rations A or B the recommended allowances can be attained with a good variety of common foods which will also provide other minerals and vitamins for which requirements are less well known. It is not safe to make this assumption for operational rations since they consist largely of processed foods. Furthermore, members of the vitamin B-complex group other than thiamine,

riboflavin and niacin should be given consideration in planning military rations. Considerable evidence has been obtained suggesting a need for additional pyridoxine in operational rations.

General Doriot has said: "It is one thing to feed a man; it is yet another to maximize the safety and efficiency of a soldier through food. Food is a munition and our objective is to maximize its military effectiveness." To implement this excellent principle we must continue the search for new information on military nutrition with the goal of describing and ultimately developing the optimum operational ration—one which will permit military personnel to carry on their activities with greatest possible efficiency.

Diet is only one pillar of good nutrition. Nutritional deficiencies may result not only from simple failure to obtain the right kind of food, but also from factors which interfere with the ingestion, absorption, or metabolism of needed nutrients, or increase the nutrient requirements. In modern global warfare military personnel are frequently exposed to various degrees of environmental physiologic and psychologic stress. Such climatic extremes as the tropics and the arctic, and stresses induced by combat, fatigue, or complete isolation, produce changes in metabolism and food requirements. Understanding of the nature and extent of such changes is basic to designing rations which will best meet the nutritional requirements of troops under these types of stress.

Additional information on the relationship of nutrition to climatic stress is needed, as a critical analysis of the available experimental and observational findings will show. Fundamental physiologic information is meager concerning the effect of diet under conditions of climatic stress on those organs and systems of the body most intimately concerned with tolerance to climatic stress. Adequate knowledge of the role of body temperature, the transfer of oxygen from the atmosphere to the tissues, and the heat economy of the human subject under these stresses has not yet been obtained.

We have some knowledge of the nutritional reactions of work fatigue, and of injury, it is true, but we know almost nothing about the interactions of these two under the emotional stress of combat further complicated by completely foreign environmental influences. Another, possibly additive, stress should also be mentioned—atomic radiation. Its effect on nutrient metabolism and nutrient requirements will require intensive study.

Because of space and weight limitations in planes and ships a high-carbohydrate food packet is the best interim solution to an all-purpose survival ration. While such a ration will support life for short periods of time, it is not optimal. The goal is a ration which will maintain the best possible state of physical well-being and enable the survivor to cope with his adverse environment until rescue takes place. With this objective in mind, special emphasis has been placed upon the feasibility of incorporating in the ration a suitable quantity of protein, high in nutritional value, to spare and replenish body tissues. This would also aid men suffering from injury, burns, loss of blood, or shock, as well as improve palatability and acceptability of the ration, promote a sense of well-being, and increase the morale of the victims of disaster.

The chief stumbling block to incorporating protein in a survival ration has always been the classic physiologic concept that protein fed at low caloric levels would perforce be burned for energy. Since the nitrogenous end products of protein thus metabolized require additional urinary water for excretion, it would be both hazardous and uneconomical to feed protein when the intake of water is limited. It has been tentatively concluded that protein can be included in a survival ration without detrimental effect, even when the energy supply is only 900 calories and water is limited to 800 cc. The suggested ration contains 900 to 1000 cal-

ories, 45 to 50 g. dried egg albumin, 30 per cent hydrogenated fat by weight and the remainder carbohydrate.

Logistic reasons make it highly desirable to have *one* all-purpose survival ration to replace five such rations now in procurement. In our current research program, considerable emphasis is being placed on confirmation and extension of these studies to climatic extremes in order to obtain guiding information for the design and development of an emergency food packet capable of sustaining life and contributing to the retention of physical stamina and mental capabilities during a period of emergency survival regardless of geographic location or climatic conditions.

Although operational rations are designed to include adequate amounts of high quality animal protein, the effect of processing and storage on the biologic value of the protein may change the picture. We need to know what the values are when the ration is eaten by the soldier in the field. Amino acid values as commonly determined by microbiologic and chemical methods may not reveal the true biologic value since acid hydrolysis masks the damaging effect of processing and does not give us a true account of the availability to the body of the component amino acids. A rapid method for the determination of the biologic value of protein, involving the use of the animal microorganism *Tetrahymena geleii* W, in which nutritional requirements for amino acids have been found to approximate those of some mammals, promises to give us a much more satisfactory means of determining the biologic value at the time of actual consumption.

Considerable losses of vitamins A and C which are sensitive to oxidation, and of thiamine which is sensitive to heat, take place during processing and storage of ration items. Processing losses of thiamine in canned food items have been found to run from 50 to 60 per cent with additional losses of 30 to 50 per cent occurring during storage for six months at 100°F. In order to provide the recommended daily allowances of vitamins at the time of consumption it is necessary to fortify operational ration items.

Most rations adequately supply vitamin C, but only because of the fortified beverage powders which they contain. Almost all of the ascorbic acid in the Individual Combat (C) and Frigid Trail rations is supplied by these fortified powders. Since many soldiers do not drink coffee, tea or cocoa, however, they do not get an adequate amount of ascorbic acid unless other fortified carriers of this vitamin are provided.

Many items which have been tested as possible vitamin carriers are not being used because they have been found to be unstable, unacceptable, or have limited utility. Needed, therefore, are stabilized forms of vitamins and/or substances which will promote the retention of vitamins added to ration items. The frequent substitutions and changes made in rations and ration components make it essential also to establish a greater number and variety of stable and acceptable fortified carriers. Work is proceeding toward this objective.

Innovations in military technics and tactics are continually adding new elements and demands to the problems of feeding personnel during combat phases of military operations. Maintaining operational efficiency through adequate nutrition under these circumstances is a challenge to fundamental and applied research. In its effort to meet this challenge the Institute is fortunate in having the guidance of the Committee on Foods, a part of the National Research Council Advisory Board on Quartermaster Research and Development Problems. This group's Subcommittee on Nutrition counsels the Institute on the special nutritive requirements of troops, on procedures for evaluating rations, areas for contract research, and on placement of

contracts. The membership of this Subcommittee includes medical nutritionists with wartime experience under the Surgeon General. With these resources for present and future planning our ability to make our armed forces equal to the demand of swift moving, highly mechanized warfare is greatly improved.

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HEAT STRESS AND FOOD INTAKE BY INFANTS

High environmental temperature has been commonly recognized as a factor suppressing food intake in adults. This has also been recognized in some species of animals, but until recently little effort has been made to study this phenomenon in human infants. Previous investigations by R. E. Cooke, E. L. Pratt, and D. C. Darrow (*Yale J. Biol. Med.* **22,** *227 (1950)*) of the metabolic response of infants to heat stress have demonstrated a tendency for sodium and chloride to be retained by normal infants during high temperature exposure unless the water intake is adequate. These studies have now been extended by Cooke (*Ibid.* **24,** *334 (1952)*) to include the alterations of food and water intake observed in infants during periods of exposure to elevated temperature.

Using the ad limitum feeding method described in *J. Am. Dietet. Assn.* **22,** *602 (1946)* 6 infants 14 to 231 days old were observed under conditions of controlled temperature, sound and lighting for twenty- to one hundred and eighty-day periods. During alternate intervals of temperature at 81°F. and at 91°F. with humidity maintained constant at 40 to 50 per cent the subjects were offered water prior to the regular milk mixture feeding. When the water was rejected, the milk mixture (milk, sugar, water and vitamins averaging 0.8 calories per milliliter) was then substituted. Intakes of the two fluids were measured for each twenty-four-hour period.

Intake during thirty-five periods of study at 81°F. averaged 120 calories (90 to 154) per kilogram of body weight per day. At the higher temperature, however, caloric consumption fell to an average of 105, although the author does not give individual figures for all subjects. This decrease in intake was reversed when the temperature was lowered. These alterations appeared to be less marked in early infancy than in more mature stages of growth and one example is given of a subject studied at 23 to 43 days of age and again at 141 to 157 days. No change occurred in the first study, but a decrease of approximately 20 per cent in caloric consumption was observed during the second period when the infant was older.

Water ingestion changed inversely with caloric intake in these subjects during hot periods. At 81°F. the water intake averaged 97 ml. (14 to 302 ml.) per kilogram per day while at 91°F. the average was 168 ml. (32 to 347 ml.). As in the case of calories, this was reversed when the temperature was lowered. Likewise, more marked alterations were observed as the infants increased in age. Although there were shifts from milk mixture to water ingestion during elevated temperature periods, the total quantity of fluid taken showed no significant increase. Intake of liquids averaged 1016 ml. per day at 81°F. and 1013 ml. at 91°F. This indicated a preferential shift to water ingestion rather than a compensatory increase in water drinking on the part of the infants during intervals of increased fluid loss through evaporation. In regard to the evaporation of fluid from body surfaces the author noted a shift in body position during heat periods which consisted of changing the predominant position to one of extension of

the extremities widely away from the body. This suggested an attempt to increase loss of body heat by increasing the amount of body surface exposed.

In an attempt to explain the phenomenon observed, gastric motility studies were carried out in 2 infants. Using the method of following the egress of radio-opaque material from the stomach, no change was found during the different temperature periods. Among the possible physiologic results effected by this adjustment of food and fluid intake, the author suggests that infants obtain a reduction in the specific dynamic action resulting from food ingestion, reduce the osmotic load of nitrogen metabolites requiring renal excretion and accompanying water loss by the urinary tract (thus conserving fluid for extrarenal loss), and reduce the sodium and chloride load taken in with the food, thus preventing excessive retention of these electrolytes and hyperosmolarity of body fluids.

In the opinion of the author these studies do not determine whether the differences in reaction of newborn and older infants represent learned behavior or the maturing of homeostatic mechanisms. Despite the questions left unanswered for the present, however, this type of investigation is of value in regard to the practical feeding of young infants. As the author suggests, the use of more dilute feeding mixtures may be indicated during periods of hot weather, particularly for newborn infants. An obvious alternative would seem to be the offering of water prior to the giving of caloric mixtures to all infants exposed to high temperature environments.

B-VITAMIN EXCRETION IN MAN AS INFLUENCED BY STREPTOMYCIN

It has been demonstrated many times with experimental animals that intestinal microorganisms can modify the nutritional value of the diet for the animals. Sulphathalidine additions to the diet of man have been reported to decrease the fecal excretion of folacin and biotin, but to have little or no effect on the urinary excretion of B-vitamins (W. E. Grundy *et al.*, *Arch. Biochem.* **15,** *187 (1947)*).

The improvement in nutritional status that was noted in rats when antibiotics were fed with diets that were partially deficient in B-vitamins has generally been assumed to be the result of changes in intestinal microorganisms (*Nutrition Reviews* **10,** *107 (1952)*).

Recently H. P. Sarett (*J. Nutrition* **47,** *275 (1952)*) has studied the effect of oral streptomycin in man on the urinary excretion of B-vitamins. It was assumed that any detectable changes resulting from such treatment could be attributed to modified intestinal bacteria.

The 6 subjects used in the study were free of organic disease and were kept in a separate metabolism ward under close dietary supervision. Each subject was on a different diet and all diets appeared adequate, as the following calculated vitamin contents indicate. The daily thiamine, riboflavin and niacin intakes ranged from 0.9 to 1.6, 1.5 to 3.1, and 9 to 17 mg. respectively in the 6 subjects. Each subject served as his own control in that vitamin excretion data were collected before and after streptomycin administration. Three of the subjects received four one-gram doses daily of streptomycin (calcium chloride complex) and the other 3 received 6 g. daily in one-gram doses.

The latter dose level was generally accompanied by a marked decrease in biotin excretion and a small decrease in the excretion of niacin metabolites (N-methylniacinamide and pyridone) within ten days. The excretion of thiamine and riboflavin

was not significantly influenced by the streptomycin administration and in one subject tested pyridoxine excretion was likewise not affected.

Folacin excretion was not decreased in any of the subjects by streptomycin administration and in 2 subjects there was an increase in the urinary excretion of this vitamin. This latter effect was considered to be related to or to serve as an explanation for the growth-stimulating action of antibiotics, which has often been reported in experimental animals.

The excretion of niacin metabolites following the feeding of tryptophan was not affected by the oral feeding of streptomycin. This was interpreted by the authors as an indication that the main conversion of tryptophan to niacin takes place in the body and not in the intestinal tract, a conclusion also reached by others (L. M. Henderson and L. V. Hankes, *Proc. Soc. Exp. Biol. Med.* **70,** *26 (1949)*; S. E. Snyderman, K. C. Ketron, R. Carretero, and L. E. Holt, *Ibid.* **70,** *569 (1949)*; W. W. Hurt, B. T. Scheer, and H. J. Deuel, *Arch. Biochem.* **21,** *87 (1949)*).

In 2 of the subjects in Sarett's studies the starch (70 and 100 g. respectively) in the diet was replaced by an equal amount of sucrose for five to ten days to determine whether or not the nature of the dietary carbohydrate would have any influence. This change had no influence on the urinary excretion of B-vitamins although it was recognized that the time intervals may have been too short to expect any profound changes in the intestinal bacteria.

Limited data were obtained regarding the changes in intestinal microorganisms brought about by streptomycin administration. Fecal cultures showed either complete or partial inhibition of the growth of coliform organisms and almost complete absence of other aerobic organisms studied (*Streptococcus viridans* and *Staphylococcus albus*).

Although the studies of Sarett were conducted on only a limited number of subjects and for relatively short periods (eight to twenty-two days of streptomycin administration), the results suggest that certain of the B-vitamins, biotin and niacin in particular, may be furnished under normal conditions to some extent by intestinal bacteria in man.

The use of antibiotics may disturb the normal intestinal flora and eliminate this source of B-vitamins to the host. Some evidence has been reported (*Nutrition Reviews* **9,** *283 (1951)*) that the extensive use of antibiotics may result in nutritional deficiencies in man.

NUTRIENT INTAKE AND SERUM LEVELS

Serum levels and nutrient intake of ascorbic acid, vitamin A and carotene in school children were studied recently by S. B. Merrow *et al.* (*J. Nutrition* **46,** *445 (1952)*). Nutrient intake and blood serum levels are frequently determined in nutrition studies of population groups (*Nutrition Reviews* **8,** *108 (1950)*). These data were collected by methods fairly typical of population studies. The practical value of such information is its use in diagnosis, treatment, and prevention of malnutrition. This study reveals the relationship between intake and serum levels in selected groups of Vermont children in 1946, 1947 and 1948.

Subjects were 160 male and female children, aged 8 to 15, selected from pupils attending public and parochial schools of Burlington, Vermont. All 160 subjects showed the anatomic changes usually related to deficiencies of vitamin A, riboflavin, niacinamide, and/or ascorbic acid. They

were divided into two groups: (1) therapy group, to study therapeutic effects of a single vitamin upon blood chemistry and lesions; (2) nontherapy group as controls. The control data are limited to some members of the nontherapy group.

Repeated blood and nutrient determinations were done on nearly all subjects over the three-year period. Seven or eight blood determinations were done on 98 per cent of the subjects. Six to nine twenty-four-hour food intake records were obtained from 80 per cent of the subjects. When data for the entire control group were examined, nutrient intake and serum levels did not correlate. Therefore further selection was made of subjects with either (1) low or high serum levels, or (2) low or high nutrient intake. These terms are purely relative, referring only to these children's data.

Serum levels were determined by the microchemical technics of O. A. Bessey, O. H. Lowry, M. J. Brock, and J. A. Lopez (*J. Biol. Chem.* **166,** *177 (1946)*). Studies were done in winter, spring, and fall; none in summer. Nutrient intakes were calculated with use of standard tables of the Bureau of Human Nutrition and Home Economics (*U. S. D. A. Misc. Pub.* **572,** *Washington, D. C. (1945)*). A report of foods eaten during the preceding twenty-four hours was secured by interview with each subject. An estimate of food ingested was facilitated by using models of dishes and utensils used by the subjects.

Low and high serum groups were selected according to scales of blood levels of O. A. Bessey and O. H. Lowry ("Meals for Millions," *Nutritional Assay of 1200 New York State School Children, p. 167, New York State Joint Legislative Committee on Nutrition (1947)*). Distributions, ranges and medians of serum levels of groups having high and low serum values for ascorbic acid, vitamin A and carotene are presented. Moderate overlapping of high and low groups occurred in distributions, ranges and median values of serum levels of vitamin C, but over-all differences are apparent. Standard deviations are not presented. The groups in which serum levels of vitamins were studied contained as few as 10 subjects and not more than 19. Apparently these subjects alone were not used for the dietary intake studies, since subjects in the dietary history, or nutrient intake groups numbered 18 to 37.

Another grouping was made on the basis of high and low nutrient intake of (1) ascorbic acid, (2) vitamin A plus carotene, (3) carotene alone, and (4) vitamin A alone. Animal food was assumed to contain only vitamin A and plant food to contain only the vitamin A-active carotenoids. Distributions, ranges and medians of nutrient intake of vitamins in these (high and low) groups show some overlapping. Differences, however, are apparent. Evidently more subjects gave dietary histories than gave blood samples for study, since groups reporting serum levels contained fewer subjects than groups reporting nutrient intake. Probably only those subjects whose blood data were available should have been included in the dietary evaluation. The purpose of using these extra dietary histories is not made clear by the authors.

In ascorbic acid studies 70 per cent of the individuals in the high group (*i.e.*, median serum level of 1.34 mg. per cent) had a daily nutrient intake of 40 mg. or over, while of the low group (*i.e.*, median serum level 0.32 mg. per cent) only 33 per cent had such an intake of ascorbic acid.

In vitamin A plus carotene studies 76 per cent of the individuals in the high group (*i.e.*, median serum level of 43 micrograms per cent) had a daily intake of 3000 I.U. or more of vitamin A plus carotene, while in the low group (*i.e.*, median serum level of 20 micrograms per cent) there were 54 per cent with such an intake.

In vitamin A studies 32 per cent of the high group (*i.e.*, median serum level of 43

micrograms per cent) had a daily intake of 3000 I.U. or more, while the low group (*i.e.*, median serum level of 20 micrograms per cent) had 29 per cent of its individuals with such an intake. Thus, intake of vitamin A alone was not reflected in vitamin A serum levels.

In carotene studies 40 per cent of the high group (*i.e.*, median serum level of 155 micrograms per cent) had a daily intake of 2000 I.U. or more, while the low group (*i.e.*, median serum level of 62 micrograms per cent) had only 25 per cent with such an intake.

Under the unusually selective conditions of this investigation and for the values shown, nutrient intakes of ascorbic acid, total vitamin A and carotene reflect serum levels of ascorbic acid, total vitamin A and carotene, respectively. Serum levels of ascorbic acid reflect intake of ascorbic acid, and levels of total vitamin A and carotene reflect the intake of those substances. Relationships were not found between serum levels of vitamin A and nutrient intake of it.

This study is limited to a select group of school children with signs of vitamin deficiencies and extremes of serum level and dietary intake of ascorbic acid, vitamin A and carotene. In a general group of school children with lesions of vitamin deficiencies serum levels could not be correlated with nutrient intake. Only by using unusually selected small groups of children were the authors able to show any relationship between serum level and nutrient intake. The child of this age group, a rapidly growing organism, probably has more need for vitamins than the mature adult. The conclusions derived from this study cannot be applied to general groups of children or to adults with deficiencies, but only to children comparable to the specific groups studied.

FATTY INFILTRATION OF THE LIVER IN NEWBORN AFRICAN BABIES

Kwashiorkor, or malignant malnutrition, is a nutritional disorder, most probably a protein deficiency syndrome, which was identified as a classic entity in Africa, and has since been recognized as one of the most prevalent deficiency diseases in the world (*Nutrition Reviews* **4,** *52, 303 (1946)*; **6,** *270 (1948)*; **8,** *55, 161 (1950)*; **10,** *261 (1952)*). It is characterized by impairment of growth, edema, lowered concentration of plasma albumin, alopecia, skin pallor, chronic diarrhea, a typical "crazy-pavement" or "flaking-paint" dermatosis and an anemia sometimes macrocytic and sometimes microcytic. At autopsy, the constant finding is massive fatty infiltration of the liver. Cystic fibrosis of the pancreas, glomerular hyalinization of the kidneys and swelling of the parotid and other salivary glands are frequent postmortem findings.

The most constant environmental factor is shortage of first-class protein, especially of animal protein, and also of "good" proteins of vegetable origin, such as beans or pulses. Plantains, cassava, sweet potatoes, millet and corn are the staple foods of the African populations among whom kwashiorkor is widespread. In recent years, it has been recognized that many conditions found in areas other than Africa, such as the "bouffissure d'Annam," an edematous syndrome of Indo-Chinese children, the fatty livers of the Brahman children and J. C. Waterlow's West Indian fatty liver disease of infants (*Med. Res. Council, London, Special Report Series* **263** *(1948)*) are all fundamentally kwashiorkor-type conditions due to poor protein nutrition.

As the onset of the visible symptoms of kwashiorkor usually follows weaning, it had been generally assumed that the pathology of the disease dates from the time when the

infant, hitherto fed maternal milk, is suddenly changed over to gruel made from the local low-protein, farinaceous staple food. W. D. Silvera and D. B. Jelliffe (*J. Trop. Med. Hyg.* **55,** *73 (1952)*), reporting on the results of liver biopsies performed on newborn babies and infants and children in the pediatric ward of University College Hospital at Ibadan, Nigeria, indicate that basic kwashiorkor lesions such as fatty infiltration of the liver and even cirrhosis may, in many cases, date back to some extent from intrauterine life.

All cases in this series were children of low income parents, peasant farmers, laborers and "petty traders," of the Yoruba tribe. One group was made up of infants under one year, many under 2 months, and several under 2 weeks of age. The other group was composed mainly of children between 1 and 3 years, with a few children up to 11 years of age. The first group was almost exclusively breast fed. The solid foods in the diets of the older children had been introduced when the children were still in part breast fed (up to 2 years of age) and consisted at weaning almost exclusively of carbohydrates (maize, yam, or cassava). Following the familiar pattern, symptoms of kwashiorkor had appeared in many of these children a few months after weaning.

As expected from the known prevalence of kwashiorkor and from the dietary histories, in all cases of the older group except one, whether the children could be diagnosed as suffering from acute malignant malnutrition or not, fatty changes were found in the livers. In the majority, the infiltration was so massive that the liver structure resembled adipose tissue. Several cases of cirrhosis were seen, with ascites and enlargement of the liver.

More unexpected was a very abnormal histologic picture seen in the younger group and, in particular, in infants under 2 weeks of age. Extreme generalized fatty changes with some necrosis was repeatedly found in the livers of apparently healthy infants. Similarly, a considerable degree of fatty infiltration was found in the livers of stillborn infants as well as of a baby who died of "blue asphyxia" on the second day of life. The extent of the fatty degeneration revealed by liver biopsies of the very young infants was in every way as profound as that observed at autopsy by J. Gillman and T. Gillman (*Arch. Path.* **40,** *239 (1945)*), H. C. Trowell (*Tr. Roy. Soc. Trop. Med. Hyg.* **42,** *417 (1949)*), and J. N. P. Davies (*Tr. Ninth Josiah Macy Conference on Liver Injury, p. 137, New York (1950)*) in typical kwashiorkor.

The authors surmise that the early, often intrauterine origin of the observed liver damage must be due to the insufficient maternal diet. The average poor Yoruba woman eats practically no animal protein, no beans, and almost exclusively starchy staple foods. The nutrition of the fetus must be handicapped by the deficient maternal dietary. Similarly, the prevalence of nutritional edema and the not uncommon occurrence of kwashiorkor in Yoruba infants still breast fed raises the possibility that in Nigerian-type nutritional situations, the composition of human milk may vary much more from Western averages than has hitherto been considered possible. Data on this important subject are surprisingly scant. Under such conditions, weaning would represent not the cause but only the precipitating factor of kwashiorkor.

Of interest in connection with this recent work is the fact that the regions where primary cancer of the liver is common are also the areas where kwashiorkor and kwashiorkor-like syndromes are prevalent (*Nutrition Reviews* **9,** *225 (1951)*). One may speculate on the etiologic relationship of early, perhaps prenatal, developmental hepatic lesions and of adult liver malignancies.

The report of Silvera and Jelliffe should impel every nutritionist to sober reflection not only because of the numerous scientific and clinical implications of the observations described by the two British authors, but

also and especially because it serves as a reminder of the miserable dietary conditions in which large parts of the world's population exist. It again emphasizes, in particular, the urgency of devising and mass-producing suitable high protein milk substitutes to be used in poor tropical areas (*Nutrition Reviews* **9,** *129* (*1951*); **10,** *261* (*1952*)).

ACUTE IRON POISONING

Iron salts have been used for many years in the treatment of disease, and particularly for the treatment of hypochromic anemia, since the introduction of Blaud's pill in 1831. About twenty years later the first reports concerning the toxicity of iron salts began to appear in the literature. However, the serious consequences of the accidental ingestion of tablets containing ferrous sulfate by children have been generally recognized only in the last five years, and the mechanisms of the acute toxicity of iron have been partially elucidated. A brief review of the recent literature reveals 24 cases of iron poisoning of which 11 have ended fatally (J. Thomson, *Brit. Med. J.* **1,** *645* (*1950*); I. O. B. Spencer, *Ibid.* **2,** *1112* (*1951*); Editorial, *J. Pediat.* **40,** *141* (*1952*).

The clinical picture is surprisingly constant. Most of the cases have occurred in children between 1 and 3 years of age who have ingested between 15 and 50 colored, candy-like tablets containing 0.2 or 0.3 g. of ferrous sulfate which had been given to some member of the family either for the treatment of anemia or following blood donation. Vomiting ensues within fifteen to sixty minutes and the vomitus often contains blood. Pallor, weakness, drowsiness and stupor rapidly ensue and the vomiting may continue. Following hospitalization and treatment for shock the child usually responds within twenty-four hours and may pass blood in his feces. In severe cases this period of apparent improvement is followed by fever, coma, often bronchopneumonia, and death less than three days after the accident. Recovery is usually uneventful and not followed by sequellae.

Pathologic findings are severe necrotizing gastritis with sloughing of large areas of the mucosa and widespread hemorrhage. Enteric coated tablets may produce similar lesions in various parts of the small intestine. S. C. Swift, V. Cefalu, and E. B. Rubell (*J. Pediat.* **40,** *6* (*1952*)) noted thrombosis of the mesenteric veins and a regional adenitis and were able to demonstrate iron in the tissues only in these areas. Widespread edema and hemorrhage, especially in the lungs, is an almost universal pathologic finding, although its etiology is not certain. Central necrosis of the hepatic lobules occurs in various degrees. The mode of death usually cannot be explained post mortem, but has generally been attributed to the toxic action of products arising from the corroded intestinal mucosa.

Spencer (*loc. cit.*) measured the serum iron concentration in 2 of his patients within four hours following ingestion of the tablets and found values of 3.3 and 3.4 mg. per cent, which are many times the normal and similar to those following the intravenous administration of iron (H. G. B. Slack and J. F. Wilkinson, *Lancet* **I,** *11* (*1949*)). An increase in urinary iron excretion has also been observed. These studies would appear to establish the fact that significant quantities of iron are absorbed despite the failure of several workers to demonstrate this substance histochemically beyond the portal vein.

Several studies on animals have confirmed the fact that it is the iron which is responsible for the poisoning. G. Forbes (*Brit. Med. J.* **1,** *367* (*1947*)) has demonstrated that the small amounts of copper and manganese salts present in the common British preparation were without toxicity

in guinea pigs and cats. The minimal lethal dose of ferrous sulfate has been determined by G. F. Somers (*Ibid.* **2,** *201 (1947)*) in mice and found to be 4.5 g. per kilogram. Blaud's pills (ferrous carbonate) were only one-fourth as toxic, corresponding to their reduced clinical effectiveness. The pathologic picture seen in guinea pigs and rabbits closely resembles that seen in the human subject.

It has been suggested by R. P. Smith, C. W. Jones, and W. E. Cochran (*New Engl. J. Med.* **243,** *641 (1950)*) that the ferritin mechanism in the intestinal mucosa is overcome or destroyed. In their case they noted that iron-containing pigment was seen only in the mucosa that was sloughing, and not in the glands which were unaffected. The absorption of large quantities of iron would then become possible.

Treatment should consist primarily of inducing vomiting as soon as possible since large numbers of undissolved pills have frequently been returned. Gastric lavage with sodium bicarbonate solutions is recommended because of the previously mentioned lower toxicity of ferrous carbonate. Intravenous fluids, plasma, or blood, and other measures to combat the shock of the initial stage are warranted. Therapy with dimercaprol (BAL) is contraindicated, since N. D. Edge and G. F. Somers (*Quart. J. Pharm. and Pharmacol.* **21,** *364 (1948)*) have shown that iron mercaptides are more toxic than inorganic iron salts. Spencer (*loc. cit.*) has advocated the use of tocopherols as antioxidants and methionine as a source of both sulfhydryl and labile methyl groups as well as large amounts of the B-vitamins in an effort to afford maximum protection of the enzyme systems which may be involved. He suggests that death following the stage of seeming improvement is due to cellular dysfunction, the liver, lungs and brain being most susceptible.

Poisoning of children by such a useful and common hematinic as ferrous sulfate is preventable. Manufacturers should be urged to indicate on the labels the dangers involved. Iron preparations must be kept out of the reach of young children.

SULFHYDRYL GROUPS AND RHODOPSIN

In a previous review (*Nutrition Reviews* **8,** *198 (1950)*) there was described the *in vitro* synthesis of rhodopsin from retinene (vitamin A aldehyde) and the protein opsin. In a recent investigation (G. Wald and P. K. Brown, *J. Gen. Physiol.* **35,** *797 (1952)*), the nature of the bond between the opsin and its chromatophore has been examined. The solutions of rhodopsin were prepared from the dissected retinas of the eyes of cattle. After the removal of interfering naturally occurring substances, the pigment was extracted with 2 per cent digitonin solution, care being exercised throughout to avoid bleaching the rhodopsin. Opsin was prepared by essentially the same procedure except that the retinas were exposed to light, thereby bleaching the pigment. Retinene was prepared from vitamin A_1 alcohol by chromatographic oxidation on solid manganese dioxide.

Either 0.1 M hydroxylamine or 0.7 M formaldehyde prevents the formation of visual purple from retinene and opsin; it appears that both reagents compete with retinene for certain combining groups on the protein. Although at first it seemed that these combining groups might be amino groups, it was finally concluded that the sulfhydryl groups on the opsin are the loci of combination. This conclusion rests on the observations that retinene acts rapidly with an excess of cysteine at pH 6.5 and that the actual synthesis of rhodopsin takes place in a pH range where combination of retinene with amino groups is minimal, but where

condensation of retinene with sulfhydryl is favored. Another proof of the main thesis is the failure of bleached rhodopsin, supplemented with retinene, to regenerate when as little as 7×10^{-4} M of parachloromercuribenzoate (PCMB), a specific sulfhydryl poison, is present in the system. Although the initial action of PCMB on proteins is reversible, this poison has no effect on rhodopsin in similar concentrations, again indicating that the sulfhydryl groups of the opsin are tied up with the chromatophore and thus protected. Sulfhydryl groups are susceptible to autoxidation, which phenomenon inhibits the rate of regeneration of bleached rhodopsin; in the presence of glutathione, however, the sulfhydryl groups of the opsin are spared to the extent of producing a definite increase in rate of regeneration of rhodopsin, further supporting evidence for the significance of sulfhydryl in this connection.

If, as appears from the foregoing observations, synthesis of rhodopsin involves the removal of free sulfhydryl groups on opsin through binding with the chromatophore, it might be expected that the bleaching of rhodopsin would be accompanied by the appearance of reactive sulfhydryl groups. This assumption was tested through the use of the amperometric silver titration for sulfhydryl groups. In this analytic device, excess silver nitrate after combining with available sulfhydryl permits a current to flow which, in turn, is indicated on the galvanometer of the system. Using this device to measure free sulfhydryl groups, rhodopsin was titrated in the dark with 0.001 N silver nitrate until a base value was obtained. Then the titration vessel was illuminated; the solution was bleached from pink to nearly colorless and the galvanometer reading dropped to half the previous value, indicating that the excess silver had been removed by the sulfhydryl groups newly liberated in the course of the bleaching of the rhodopsin.

The appearance of free sulfhydryl groups in the course of bleaching of visual purple is one of several indices which point to the similarity between this phenomenon and the denaturation of proteins, although certain observations with respect to changes in rhodopsin during bleaching render difficult a simple explanation of the structure of this pigment based on reactions involving the sulfhydryl group. In the amperotitration with silver, light produces a reaction liberating reactive groups, which, through a redistribution of ions, generates an electrical response. This analytic device may lead the way toward a better understanding of the excitation in the retina, optic nerve and brain occurring in visual sensation.

MODE OF ACTION OF THIAMINASE

In 1936 there was published an account (R. G. Green, *Minn. Wildlife Disease Invest.* **2,** *106 (1936)*) of a type of paralysis in ranch foxes which were fed whole raw fish as part of their diet (*see also Nutrition Reviews* **1,** *61, 403 (1943)*; **2,** *128 (1944)*; **4,** *74 (1946)*). It was subsequently shown (Green, W. E. Carlson, and C. A. Evans, *J. Nutrition* **21,** *243 (1941)*) that the paralysis is a manifestation of thiamine deficiency and also that the responsible agent in raw carp entrails is a heat-labile factor which destroys vitamin B_1 (E. H. Spitzer, A. I. Coombes, C. A. Elvehjem, and W. Wisnicky, *Proc. Soc. Exp. Biol. Med.* **48,** *376 (1941)*). The enzyme was later called thiaminase and, as might be expected, it was shown to exert no untoward influence if the fish does not come in contact with the vitamin B_1 of the diet (Green, Carlson, and Evans, *J. Nutrition* **23,** *165 (1942)*). The action of the enzyme is to cause a hydrolytic cleavage of the thiamine between the thiazole and the pyrimidine rings (L. O.

Krampitz and D. W. Woolley, *J. Biol. Chem.* **152,** *9 (1944)*).

Thiaminase has been found in shellfish (A. Fujita and I. Numata, *18th annual meeting, Jap. Biochem. Soc., October 7, 1942*) and also in an intestinal organism subsequently named *Bacillus thiaminolyticus* (Fujita and Numata, *19th annual meeting, Jap. Biochem. Soc., October 30, 1943*). Extensive studies of the mechanism and conditions of the thiaminase reaction have been carried out by Fujita and his co-workers. An assay procedure has been worked out in which acid aqueous extracts of whole clam, fish or bacterial culture were used as the source of the enzyme. This extract or a similar one from the sample to be assayed is allowed to react with a known quantity of thiamine at pH 5.5 for one hour and the remaining vitamin B_1 is estimated quantitatively by the thiochrome method. With this experimental device, the influence of a large number of aromatic amines, heterocyclic compounds and sulfhydryl compounds upon the activity of thiaminase has been studied (Fujita *et al., J. Biol. Chem.* **196,** *289 (1952)*).

Aromatic amines acted to stimulate the activity of the enzyme from all three sources, the most effective compound being aniline. Pyridine and quinoline produced marked activation of the enzyme. Unless the amino group was attached to the ring directly or if the amino group was substituted, no accelerating action could be demonstrated. In several of the tests the bacterial thiaminase reacted differently than did the enzyme from the other sources. Sulfhydryl compounds were effective as stimulants for the activity, particularly of the bacterial enzyme.

When pyridine was the activating agent, there frequently appeared an interfering green-yellow fluorescence which was called "heteropyrithiamine" and was shown to be a compound in which the pyrimidine was attached to pyridine instead of to the thiazole group as in thiamine (A. Fujita, Y. Nose, K. Ueda, and E. Hasegawa, *J. Biol. Chem.* **196,** *297 (1952)*). The green-yellow fluorescence was intense when thiamine, pyridine and thiaminase were in the reaction mixture, but when only the pyrimidine part of thiamine, pyridine and the enzyme were present, very weak fluorescence was observed. From these observations it was deduced that, where thiaminase acts on thiamine in the presence of pyridine, the latter replaces the thiazole part of the vitamin. By chemical synthesis and isolation, as well as by comparison of chromatographically separated fractions with the synthetic intermediates, it was concluded that the decomposition of vitamin B_1 is not a simple hydrolysis, but rather an exchange of bases, the replacement of the thiazole part of thiamine by amines present in the crude enzyme preparation. It is suggested that the thiaminase itself be looked upon as the "apoenzyme" and other bases in the crude enzyme preparation from other sources be considered the "coenzyme." The thiaminase, therefore, is considered to catalyze a base exchange reaction involving thiamine.

An effort was made to separate the "coenzyme" from the "apoenzyme" of bacterial and shellfish thiaminase (A. Fujita and T. Tashiro, *J. Biol. Chem.* **196,** *305 (1952)*). Repeated precipitation with acetone from acid aqueous extracts yields a solid which consists of the "apoenzyme." The preparation from shellfish and from bacteria is unstable even at low temperatures. This preparation is active when heterocyclic amines or aromatic amines are present as "coenzymes." If there is species specificity with respect to degree of activity, it appears to reside in the "apoenzyme" part. It was also observed that the deamination of the natural coenzyme or of the active known amines or thiols stops the activity of the enzyme system.

If the foregoing pathway of enzymatic degradation of thiamine is valid, it should be possible to demonstrate the synthesis of

the vitamin from a pyrimidyl base and 4-methyl-5-beta-hydroxyethylthiazole. Various substituted aminomethyl pyrimidines were synthesized and each incubated with thiazole and the thiaminase preparations from the three sources (A. Fujita, Y. Nose, S. Uyeo, and J. Koizumi, *J. Biol. Chem.* **196,** *313 (1952)*). The data indicate that thiamine was synthesized under these experimental conditions, although it appears that the equilibrium is far in the direction of the breakdown of the vitamin.

HEREDITARY OBESITY OF MICE

The importance of obesity as the most prevalent form of human malnutrition in this country is receiving increasing attention. It is for this reason that studies of experimental obesity in animals should be of interest. The obesity which follows hypothalamic lesions has been described by many authors. Study by J. R. Brobeck, J. Tepperman, and C. N. H. Long (*Yale J. Biol. Med.* **15,** *831 (1943)*) of obese rats following such lesions in particular has helped define the pathologic disturbance of appetite produced in these animals.

The production of a strain of mice with a high incidence of obesity has been achieved by A. M. Ingalls, M. M. Dickie, and G. D. Snell, *J. Hered.* **41,** *317 (1950)*). These animals appeared in crossings of two related strains of mice. The character of obesity becomes apparent at an early age and by the time the mice are young adults they weigh 38 to 56 g. as compared to weights of 16 to 26 g. in their nonobese siblings. The obesity appears to be due to the recessive gene "obese." In the homozygous condition the animals are sterile in addition to having the metabolic disorder. Mice heterozygous for this character are normal.

Diabetes mellitus also occurs in these mice. J. Mayer, M. W. Bates, and M. M. Dickie (*Science* **113,** *746 (1951)*) found the blood sugar of fed obese mice to be generally over 200 mg. per cent as compared to 110 mg. per cent in the nonobese members of the strain. The blood sugar fell promptly during fasting to normal levels. Glycosuria of 1 to 3 per cent occurred when the animals were allowed to feed. Of great interest was the refractoriness to insulin exhibited by these mice. In a small group of obese mice 20 units and even 400 units per kilogram of body weight of insulin were without effect on the blood sugar. Comparable doses of insulin in nonobese mice proved lethal.

The appetite of the obese and nonobese mice of this strain for high protein, high fat and high carbohydrate diets was compared by J. Mayer, M. M. Dickie, M. W. Bates, and J. J. Vitale (*Science* **113,** *745 (1951)*). Three diets were prepared in which 90 per cent of the calories was represented as carbohydrate, fat, and protein respectively. The mice were permitted to select from the three diets and their daily food consumption was measured. The obese mice consumed 5 calories per day more than the nonobese animals ($P = 0.03$), and significantly more fat, but less protein and carbohydrate than did the nonobese siblings.

The basal oxygen consumption of the hereditarily obese mice has been recently reported by J. Mayer, R. E. Russell, M. W. Bates, and M. M. Dickie (*Endocrinology* **50,** *318 (1952)*). The authors describe in detail their methods for the determination of respiratory exchanges in individual mice. Great care was exercised to insure the absorption of carbon dioxide and water vapor. It was found that the total oxygen consumption of the obese mice was slightly although not significantly less than that of their nonobese siblings. Calculated per unit of weight, the oxygen consumption of obese mice was 50 per cent that of the nonobese mice. Even when the calculations are made on the basis of surface area the obese mice

consumed 44 per cent less oxygen than their normal siblings. The respiratory quotients of the obese and normal mice of this strain were the same.

The injection of 50 micrograms of thyroxine daily for two weeks resulted in an increase in oxygen consumption from 0.0981 to 0.178 cc. of oxygen per unit of surface per minute. The normal mice increased their oxygen consumption from 0.175 to 0.202 cc. of oxygen per unit of surface per minute.

The exact cause of obesity in these mice remains to be elucidated. The observations on reduced basal oxygen consumption and the slightly increased caloric intake explain the obesity in terms of over-all balance. Possible defects in the intermediary metabolism of fat are under investigation and should contribute pertinent information on this problem. Histologic studies (V. R. Bleisch, J. Mayer, and M. M. Dickie, *Am. J. Path.* **28,** *369* (*1952*)) have revealed that hyperplasia of the islands of Langerhans is a constant finding in the obese members of the strain. No definite histologic abnormality was noted in other endocrine organs.

The relation of obesity to the disturbed carbohydrate metabolism is obscure. The association of relatively mild alimentary diabetes, obesity and insulin resistance is met frequently in clinical diabetes. It is possible that the study of the disease in mice may assist in our understanding of one type of human diabetes.

RELATION OF CEREALS AND SUGARS TO EXPERIMENTAL DENTAL CARIES

Studies on the relation of diet and nutrition to tooth decay in experimental animals could be classified in two general categories: those investigations concerned with the relation of nutrition to the development of teeth and their later susceptibility to dental caries (*Nutrition Reviews* **6,** *196* (*1948*); **8,** *37* (*1950*)), and those experiments which are primarily concerned with the relation of dietary composition to the maintenance of teeth after they are fully formed and erupted into the oral cavity (*Ibid.* **6,** *75*, *105* (*1946*); **8,** *344* (*1950*); **9,** *255* (*1951*)).

In an investigation to study the relation of cereals and sugars to the maintenance of fully developed teeth, M. A. Constant, P. H. Phillips, and C. A. Elvehjem (*J. Nutrition* **43,** *551* (*1951*)) have reported a series of experiments with cotton rats in which the cariogenicity of a diet consisting of oatmeal, dried whole milk and sugar was studied and in which the caries-producing properties of natural and refined sugars were compared. The basal diet in the first experiment consisted of a homogenized mixture of 50 g. of finely ground oatmeal with sufficient whole milk to provide 33 g. of milk solids. This aliquot of the basal diet was modified by the incorporation into it of either 17 g. of cerelose or enough of a natural syrup to supply 17 g. of sugars. All three diets were fed in liquid form throughout the experimental period of fourteen weeks. Parallel to the animals on these three diets was run a group on a dry diet consisting of 50 g. of oatmeal, 33 g. of dried whole milk powder and 17 g. of sugars prepared by drying the natural syrup. The cotton rats fed the three liquid diets had an average of only one small carious lesion per capita whereas the group fed the dry ration had an average incidence of 25 carious lesions per head and an average caries score of 72+. Obviously, the fluidity of the rations drastically reduced the incidence and extent of tooth decay. This was true even in the fluid ration which had the same percentage distribution of solids as the dry ration. This phenomenon has been reported previously in instances where purified rations were studied instead of the natural

diet used in this case (E. P. Anderson, J. K. Smith, Elvehjem, and Phillips (*Ibid.* **35,** *371 (1948)*).

In the second experiment, a purified diet was used which consisted of a sugar at 23.4 per cent, dextrin 43.6 per cent, casein 24 per cent, salt mixture 4 per cent, and corn oil 5 per cent. The sugar was either supplied as sucrose, cerelose, as a natural syrup, or as a synthetic syrup (prepared from a mixture of 20 per cent glucose and 80 per cent sucrose by heating to the approximate viscosity of natural syrups). No difference in the average number of carious lesions or in the average caries score was observed as a result of the presence of the naturally occurring versus the refined sugars. A similar inability to distinguish between the caries-producing powers of the sugar in a natural syrup and sucrose was observed when the two were incorporated at the same level into the oatmeal-whole milk powder diet.

In the third experiment, the whole milk powder in the oatmeal-whole milk powder diet was replaced by casein and dextrin in a ratio of 1:3 by weight. This manipulation resulted in a striking increase in the average number and extent of carious lesions in the cotton rats in this group. This was a particularly interesting observation in view of the fact that the amount of simple sugars in the resulting diet was quite low. A side observation of note was the comment that there was a definite trend toward rickets in this group. This occasioned the investigators to draw attention to the fact that there was a correlation between the low mineral content of the diet and the incidence of dental caries.

In the fourth experiment, the whole milk powder was decreased to 16 per cent with a commensurate increase of oatmeal, or increased to 48 per cent with a comparable decrease in oatmeal. These lesser modifications of the milk and oatmeal content of the diet did not affect the incidence and extent of tooth decay to a significant degree.

In a later series of studies, M. A. Constant, P. H. Phillips, and C. A. Elvejhem (*J. Nutrition* **46,** *271 (1952)*) have reported on the relation of whole grain and processed cereals to the production of tooth decay in cotton rats. The cariogenicity of a purified diet containing 67 per cent sucrose, 24 per cent casein, 4 per cent salt mixture, 5 per cent corn oil, and 4 per cent of 1:20 liver extract was compared with the caries-producing power of a diet composed of 50 per cent oatmeal, 32 per cent whole milk, and 18 per cent sucrose. The average incidence of carious lesions was 11.0 for the purified ration and 18.5 for the oatmeal basal ration. The average extent of carious lesions was 24+ for the cotton rats fed the purified ration and 54+ for those fed the oatmeal basal diet. This was a striking observation in view of the fact that the oatmeal basal diet contained only 30 per cent sugars (18 per cent sucrose and 12 per cent lactose).

In the second experiment, the oatmeal in the oatmeal basal diet was replaced entirely by whole corn or corn flakes, whole wheat or wheat flakes, and whole oats. Each of these materials was finely ground in order that there would be no effect of particle size on the caries incidence. It was found that in each case the whole grains were about 50 per cent less cariogenic than the comparable processed cereal product.

In the third experiment, various manipulations were made to change the ratio of alkaline and acid elements in the ash content of the diet. The basal ration in this case consisted, in per cent, of: casein, 17; celluflour, 5; floured dextrin, 53; sucrose, 18; salts IV, 2; and corn oil, 5. Various cereals were used in place of the dextrin in the basal diet. Floured potato was used as an alkaline ash food. Wheat flour, rice flour and finely ground oatmeal were used as representative of acidic ash foods. The character of the potato flour was altered by the addition of sufficient 1 Normal hydrochloric acid to provide an excess of 0.1 equivalent of acid

per 100 g. of cereal. The acidic ash of the rice and oatmeal was altered by the addition of sufficient 1 Normal sodium hydroxide to provide an excess of the same amount of base. These materials were then dried and reground to the same particle size as the original samples. When the floured potato was compared with the floured potato with added acid in place of dextrin, neither altered the incidence or the extent of carious lesions. Likewise, when oatmeal or oatmeal plus base, wheat flour, floured rice, or floured rice plus base were used in full replacement of dextrin, none of them significantly altered the incidence or the extent of carious lesions. Thus altering the ash characteristics of the diet by the use of these foodstuffs instead of dextrin did not alter the cariogenicity of the ration, nor did the addition of acid or base to the cereals alter their cariogenicity.

The high caries-producing properties of these low-sugar cereal diets are particularly interesting. Obviously, there are other factors besides the sugar content of the ration which contribute to the caries-producing properties. The fact that sugar in naturally occurring syrups had the same caries-producing properties as the same percentage of refined sugars is an answer to the often quoted statements that sugars in their natural state are not cariogenic. Another interesting point is the higher caries-producing properties of processed cereals than the raw grains. It would be valuable to know what the change has been which resulted in this alteration. Perhaps the hulls of whole grains may carry something which may help protect against dental caries.

THE RETICULOENDOTHELIAL SYSTEM IN VITAMIN A METABOLISM

In the nephrotic syndrome of children, B. M. Kagan, E. M. Thomas, D. A. Jordan, and A. F. Abt (*J. Clin. Invest.* **29,** *141 (1950)*; *Nutrition Reviews* **8,** *189 (1950)*) have found that there is an elevation of the fasting serum vitamin A level and that the level of vitamin A rose much higher than normally after the oral administration of 6000 U.S.P. units per pound of body weight. The fasting serum carotene concentration was found to be normal. These findings suggested to the authors that patients with the nephrotic syndrome failed to store or utilize vitamin A. An impairment in the liver cells, perhaps related to protein depletion, was suggested.

Prompted by the changes in vitamin A metabolism in nephrosis, H. Z. Brown, I. Philips, and B. M. Kagan (*Metabolism* **1,** *349 (1952)*) have restudied the role of the reticuloendothelial system in vitamin A metabolism. The possible significance of the Kupffer cells in vitamin A storage was suggested by the experiments of F. Lasch and D. Roller (*Klin. Wchnschr.* **15,** *1636 (1936)*) which demonstrated that the ability of the liver to take up vitamin A could be abolished by the blockade of the reticuloendothelial system with trypan red and thorium dioxide. When, however, the parenchymal cells were poisoned by phosphorus, vitamin A could still be stored in the liver. Although these conclusions were supported by other workers, contradictory results were obtained by L. S. Bentley and A. F. Morgan (*J. Nutrition* **31,** *333 (1946)*), who employed trypan blue alone.

In the present experiments guinea pigs were given three daily intraperitoneal injections of trypan red and thorium dioxide. On the day after the above preparation had been completed 6000 U.S.P. units of vitamin A palmitate per pound of body weight were given by stomach tube. Blood samples were obtained at three, six and twenty-four hours after administration of the vitamin. The control guinea pigs which had not received the trypan red and thorium dioxide had an initial serum vitamin A level of 21.9 micro-

grams per cent. After vitamin A administration the level rose to 363, 193 and 33.7 micrograms per cent at three, six and twenty-four hours respectively.

In the animals with reticuloendothelial blockade the initial vitamin A level was 20.5 micrograms per cent, a value almost identical to the control levels. Much higher levels of vitamin A were reached in the experimental group after the vitamin was given. The levels averaged 1287, 1375 and 282 micrograms per cent three, six and twenty-four hours after the dose. The authors made the incidental observation that the serum cholesterol was 49.9 mg. per cent in the guinea pigs treated with trypan red and thorium dioxide as compared to the control level of 16.7 mg. per cent in the normal animals. No change in the ratio of cholesterol to cholesterol esters took place in the treated animals.

The histologic appearance of the livers of the two groups was identical except that the livers from the treated guinea pigs showed fine particles within the Kupffer cells. No evidence of damage to the parenchymal cells was found.

These results are further support for the theory that the reticuloendothelial system may have particular importance in the removal of vitamin A from the blood. It has not been established that this mechanism is significant under conditions of normal vitamin A intake. The relevance of the experimental results to the abnormality of vitamin A metabolism in nephrosis is not clear. In the experiments of Lasch and Roller (*loc. cit.*) the liver content of vitamin A was found to be low. In nephrosis, however, B. M. Kagan and E. Kaiser (*personal communication*) have found in as yet unpublished analyses that the vitamin A level of the liver is actually elevated. The theory which Brown *et al.* (*loc. cit.*) present is that the reticuloendothelial system may in nephrosis be saturated with vitamin A and therefore cannot take up additional vitamin A.

The functional disturbance was compared to hypervitaminosis A in that additional vitamin A in this condition causes excessively large increases in blood level. Although the theory may be an adequate explanation of the alterations in blood levels, it fails to explain why the reticuloendothelial system should be saturated in nephrosis. A defect in disposal or an increased production of the vitamin from precursors may still be the fundamental metabolic disturbance.

EFFECT OF CHOLINE AND CYSTINE ON METHYL GROUP OXIDATION

It has long been known that methyl groups can be lost to the organism by excretion as components of creatinine, creatine, and N′-methylnicotinamide (*Nutrition Reviews* **8,** *269* (*1950*)). It has also been shown (C. G. Mackenzie *et al.*, *J. Biol. Chem.* **180,** *99* (*1949*)) that when methionine labeled with C^{14} in the methyl group is fed to rats, an important fraction of the radioactivity can be recovered as expired carbon dioxide during the first twenty-four hours. The loss continues later at a slower rate. Mackenzie and his collaborators further showed (*Ibid.* **183,** *617* (*1950*)) that the rate of oxidation of the methyl group is markedly increased when the level of methionine in the diet is increased. Since methionine is known to be a source of the methyl group of choline (*Nutrition Reviews* **2,** *358* (*1944*)) and of the sulfur of cystine (W. C. Rose, *Science* **86,** *298* (*1937*)) it seemed logical to examine the effect of these two substances on the rate of oxidation of the methyl group of methionine.

To this end C. G. Mackenzie and V. du Vigneaud (*J. Biol. Chem.* **195,** *487* (*1952*)) fed young male rats for ten weeks four experimental diets, one basal diet devoid of choline and cystine, and others supplemented with either or both of these compounds. The

basal diet contained 0.6 per cent methionine, which is equivalent to the amount provided by a 17 per cent casein ration. Methionine in the basal diet was thus the exclusive source of methyl group and of sulfur. Growth was satisfactory on all diets, with no fatty infiltration of the liver or kidney damage observed. At the end of the ten-day period, each rat received by stomach tube a 2-gram portion of its respective diet in which methionine, labeled with C^{14} in the methyl group, replaced the nonradioactive methionine previously fed. The animal was then placed in a respiration chamber, and the radioactive carbon eliminated in the expired carbon dioxide, urine and feces during the next twenty-four hours was collected and determined.

Under the conditions of the experiment it was found that the rats fed the unsupplemented basal diet eliminated during the twenty-four-hour period 3.5 per cent of the methyl group radiocarbon as expired carbon dioxide. The rate of oxidation rose during the first three hours and declined during the next three hours to a stable level indicative of a relatively steady rate. The first six-hour period during which the increase and decrease in the rate of oxidation take place is termed by the authors the "period of assimilation"; the stable period which succeeds it they call the "period of equilibrium." The effect of adding choline to the basal diet on the rate of oxidation of the methyl groups of methionine was striking. The rate of oxidation was increased ten-fold during the first hour by the addition of 0.2 per cent choline chloride to the diet. The total increase during the twenty-four-hour period was three-fold. When both cystine (0.4 per cent) and choline were added to the basal diet, the rate of oxidation was restored to the level characteristic of the basal diet. Finally, the addition of cystine alone did not alter, and in particular did not decrease, the oxidation rate corresponding to the basal diet. The excretion of radiomethyl carbon in the feces or the urine was the same on the basal and on the supplemented diets.

An explanation of the reported findings is suggested by consideration of this experiment as a situation where cystine is first removed from a complete diet containing methionine, choline and cystine. The absence of cystine then results in a great increase in the oxidation of the methyl groups, presumably because methionine is being used as a source of sulfur in the synthesis of cystine. According to Mackenzie and du Vigneaud, the further withdrawal of choline makes methionine the "sole source of exogenous labile methyl groups." While there is evidence that some biologic synthesis of methyl groups does take place (*Nutrition Reviews* **8,** *269 (1950)*; **9,** *231 (1951)*), these authors do not mention the possibility that it would modify their interpretation. Viewed from this standpoint the experiment of Mackenzie and du Vigneaud illustrates in a striking manner the complexity of the metabolic picture existing in multiple partial deficiencies. Such a picture cannot be arrived at by a simple addition of the effects of single partial deficiencies. It is even possible that, as in the example considered here, a normal rate of oxidation or of excretion of a metabolite is the result of two deficiencies which are in this one respect mutually compensating.

VITAMIN B_{12}, STREPTOMYCIN AND ENERGY METABOLISM

Much evidence has accumulated to indicate that vitamin B_{12}-antibiotic supplements, when added to practical rations for chickens and swine, will stimulate growth and usually will also result in more efficient feed utilization for growth (*Nutrition Reviews* **8,** *298 (1950)*; **9,** *359 (1951)*).

A careful study of the energy and nitrogen

metabolism as influenced by vitamin B_{12} and streptomycin was undertaken by A. Black and J. W. Bratzler at Pennsylvania State College (*J. Nutrition* **47,** *159 (1952)*). Weanling albino rats were used. The basal ration used had the following percentage composition: soybean oil meal, 30.0; linseed oil meal, 12.24; salt mixture, 4.0; corn oil, 5.0; dextrin, 24.38; and cerelose, 24.38. Vitamin supplements consisted of the following (milligrams per kilogram of diet): oleum percomorphum, 570; mixed tocopherols, 150; choline, 4000; inositol, 2000; folacin, 0.025; biotin, 0.010; thiamine hydrochloride, 5; riboflavin, 5; pyridoxine hydrochloride, 6.25; niacin, 6.25; calcium pantothenate, 50; para-aminobenzoic acid, 95; and 2-methyl-1,4-naphthoquinone, 2.5.

Two separate experiments were conducted. In the first experiment the basal diet and the basal diet plus a commercial vitamin B_{12} supplement containing streptomycin were compared both under ad libitum and pair-feeding conditions. In the second experiment the component parts, namely the vitamin B_{12} and the streptomycin, were fed separately to two groups of rats and together to a third group. The vitamin B_{12}-antibiotic supplement supplied 33 micrograms of vitamin B_{12} and 5.5 mg. of streptomycin per kilogram of diet.

When the basal and the supplemented diet were fed ad libitum (first experiment), the rats on the supplemented diet attained the final weight of approximately 220 g. in thirty-five days whereas those on the basal diet required sixty-nine days to attain the same body weight. When pair-fed, those on the basal diet determined the feed intake of those on the supplemented diet in almost every case.

Of particular interest were the observed changes in body composition brought about by the vitamin B_{12}-antibiotic supplement. Rats on the basal diet gained 163 g. in sixty-nine days, and of this gain, 5.5 g. were nitrogen and 15.4 g. were fat. Pair-fed rats receiving the supplement gained 182 g. in sixty-nine days, 6.1 g. of which were nitrogen and 24.1 g. of which were fat. The most striking effect of the supplement was the increase in gain of body fat with only a slight increase in gain of body nitrogen (protein).

The following three phases of energy metabolism were noted by the Pennsylvania workers to be influenced by the vitamin B_{12}-antibiotic supplement: (1) The metabolizable energy (food energy corrected for energy in feces and urine) was 2586 calories for the supplemented rats and 2561 calories for the pair-fed controls during the experimental period. (2) Body gain of energy was 442 calories for the supplemented animals compared with 342 calories for the controls. (3) Heat production was 2144 calories in the supplemented and 2219 in the control animals. All of these were highly significant differences and point to the fact that the supplement exerted a real and marked influence in favor of improved energy metabolism.

The most striking effect of supplement feeding was obviously its influence on appetite. Animals fed the supplemented diet ad libitum attained the final body weight of approximately 220 g. on 492 g. of food in thirty-five days, whereas those on the unsupplemented ration consumed 754 g. of food to attain the same weight in sixty-nine days. The authors thus feel that perhaps the most important cause for increased gains and feed efficiency that are usually obtained in practical rations for such supplements is the appetite stimulation.

The results of the second experiment indicated that nearly all of the response from the commercial vitamin B_{12}-antibiotic supplement was attributable to its vitamin B_{12} content. For example, rats fed the basal diet plus streptomycin required eighty-two days to reach the arbitrary final body weight of 260 g., those fed the basal diet plus vitamin B_{12} required only forty-one days to attain this weight, whereas those fed both vitamin B_{12} and streptomycin required thirty-nine

days. As in the first experiment the supplement of vitamin B_{12} had a significant influence on energy metabolism and approximately 6 per cent less of the food energy was lost as heat, when compared with the group not receiving vitamin B_{12}.

Black and Bratzler point out that their results are in agreement with those of others who have reported a vitamin B_{12} influence on protein utilization (E. L. Hove and J. O. Hardin, *Proc. Soc. Exp. Biol. Med.* **77,** *502 (1951)*; A. M. Hartman, L. P. Dryden, and C. A. Cary, *Arch. Biochem.* **23,** *165 (1949)*). The Pennsylvania workers have, however, made the added observation that protein metabolism is no more directly associated with vitamin B_{12} than is over-all growth and particularly over-all energy utilization.

From these results it is probable that much of the benefit gained from vitamin B_{12}-antibiotic supplements in swine and poultry rations is effected through the influences of such supplements on over-all energy metabolism.

CHOLESTEROL IN EXPERIMENTAL HYPERTHYROIDISM AND HYPOTHYROIDISM

Clinical experience has demonstrated an effect of thyroid hormone on plasma concentrations of cholesterol. In general, hyperthyroidism is associated with a decrease and hypothyroidism with an increase in total plasma cholesterol. A recent review was concerned with the changes in plasma and tissue cholesterol occurring with the feeding of powdered thyroid substance to young rats. It was pointed out that in addition to the expected decrease in plasma cholesterol there was also a decrease in the cholesterol and total lipid content of the liver (*Nutrition Reviews* **10,** *208 (1952)*).

The mechanism of action of thyroid hormone on cholesterol metabolism has been investigated. Earlier reports have indicated that the liver contributes significantly to the cholesterol of plasma and also is effective in removal of plasma cholesterol through biliary excretion (S. O. Byers, M. Friedman, and F. Michaelis, *J. Biol. Chem.* **188,** *637 (1951)*; Friedman, Byers, and Michaelis, *Am. J. Physiol.* **164,** *789 (1951)*). In an effort to determine whether or not thyroid hormone influences the excretory loss of cholesterol in bile, the quantity of cholesterol excreted in bile of normal rats was compared with that of rats with deranged thyroidal function (R. H. Rosenman, Friedman, and Byers, *Circulation* **5,** *589 (1952)*). The rats utilized were young male rats fed a usual stock diet. Hyperthyroidism was induced in one group of animals by including powdered thyroid substance in the diet at a level of 0.12 or 0.30 per cent. Other animals were made hypothyroid by feeding thiouracil, in concentrations of either 0.25 or 0.30 per cent of the diet. Rats in the two groups were maintained on the specific regimen for periods of eighteen or forty-two days. Control animals were fed the stock diet without supplements of thyroid or thiouracil. At the end of the feeding period blood was withdrawn for determination of plasma cholesterol and the bile duct of each animal was cannulated. The total quantity of bile excreted in a twenty-four-hour period was collected. All food was withheld during this period. The total quantity of cholesterol excreted was determined.

Signs of hyperthyroidism and hypothyroidism appeared in the rats fed thyroid and thiouracil, respectively. The total plasma cholesterol changed in the directions anticipated. The mean cholesterol of plasma of control animals was 54 mg. per cent, which compared with a value of 40 mg. per cent in rats fed thyroid powder and 67 mg. per cent in hypothyroid animals.

The volume of bile excreted by animals in the three groups was the same, 12 to 13

ml. per twenty-four hours. Concentrations of cholesterol in bile varied widely, however, and in directions opposite to those of plasma cholesterol. Mean concentrations of bile cholesterol were 21 mg. per cent in the control group, 42 mg. per cent in the hyperthyroid group, and 10 mg. per cent in hypothyroid animals. Calculations of total biliary excretion of cholesterol indicated that hyperthyroid animals lost twice as much cholesterol by this route as did control rats, while the loss of hypothyroid animals was but half that of the controls. Groups of animals were large enough and analyses of variance such that these differences are shown to be significant by statistical tests. These observations would seem to indicate that the thyroid hormone has a direct influence on the excretion of cholesterol in bile. The lower concentrations of cholesterol in plasma in the hyperthyroid state may be in part at least the result of increased loss of cholesterol in bile, while the accumulation of plasma cholesterol in hypothyroidism may result from decreased rates of excretion of biliary cholesterol.

The same investigators attempted by other means to determine if other mechanisms also contribute to the changes in plasma cholesterol in deranged thyroidal function. It had been observed that following experimental total obstruction of biliary flow cholesterol accumulated in plasma at a relatively constant rate. To determine if states of hyperthyroidism or hypothyroidism have an effect on this rate of accumulation of cholesterol, plasma levels were determined twenty-four hours after obstruction of the bile duct. Three groups of rats prepared as in foregoing experiments were used. There was more rapid accumulation of cholesterol in plasma of hyperthyroid rats than in control animals following biliary obstruction, the net increase being 50 per cent greater in twenty-four hours. In contrast, the increase in plasma cholesterol of hypothyroid rats was only 70 per cent that of the control groups. From these findings it would appear that the thyroid hormone has a role in conditioning the release of cholesterol into the plasma, presumably from the liver, or is concerned in processes of degradation of plasma cholesterol in such a manner that hyperthyroidism permits release of or accumulation of cholesterol in plasma of animals with biliary obstruction.

Presumptive evidence indicating that the predominant effect of the hormone is one of effecting an increased release of cholesterol from the livers of rats with biliary obstruction was obtained by raising the concentration of plasma cholesterol by injection of hypercholesteremic plasma. In hyperthyroid animals without biliary obstruction the injected cholesterol was totally removed within six hours, while periods of more than twelve hours were required for the removal of like amounts of injected cholesterol in hypothyroid and control groups. It is to be regretted that the investigators did not compare the biliary excretion of cholesterol in these animals following the artificially induced hypercholesteremia.

In another report the same investigators describe the results of experiments in which the fecal excretion of cholesterol and related sterols have been determined in rats with similarly induced deranged thyroid function (M. Friedman, S. O. Byers, and R. H. Rosenman, *Circulation* **5,** *657 (1952)*). After eight weeks on the thyroid- and thiouracil-supplemented diets, rats were placed on sterol-free diets and the feces were collected over a seventy-two-hour period. The animals were sacrificed and the intestinal contents added to the fecal specimens. Specimens were dried, weighed, and extracted with lipid solvents. The cholesterol and noncholesterol sterols were measured after precipitation with digitonin. It was found that both the fecal cholesterol and noncholesterol sterol were significantly greater in the hyperthyroid rat than in control animals. In hypothyroid rats the total fecal sterol excretion was significantly less than in controls, with a greater reduction in noncholesterol sterol than in

cholesterol. These findings would seem to confirm the observations made by cannulation of bile ducts.

In reviewing these experiments it must be concluded that they provide important new information concerning mechanisms of action of thyroid hormone on cholesterol metabolism. The hormone is demonstrated to have at least two actions, one in control of hepatic synthesis and release of cholesterol into plasma, another in influencing rates of biliary excretion of cholesterol. The second of these would seem to be the predominant one, since despite increased release of cholesterol, plasma concentrations are maintained at levels lower than normal. It may be that the increased rate of synthesis in the liver results from the stimulus of more rapid loss of cholesterol in bile. Other factors may contribute also to the decreased plasma cholesterol in hyperthyroidism. For instance, little is known concerning the metabolism of cholesterol in other tissues of the body in this state. It is entirely possible and perhaps probable that degradative reactions involving cholesterol proceed at increased rates in many organs and tissues of the body.

NIACIN AND DIPHOSPHOPYRIDINE NUCLEOTIDE

Biologic activities of certain of the vitamins are dependent on conversion to metabolically active derivatives or coenzymes. Thus, the water-soluble vitamin, niacin, is a precursor in the biologic synthesis of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), or coenzymes I and II. Many organisms require the preformed vitamin to achieve synthesis of pyridine nucleotides. However, exceptions exist. Certain of the molds and bacteria produce niacin from carbon dioxide and ammonia. Mammals also may utilize an alternative source through synthesis of the vitamin from the amino acid tryptophan (*Nutrition Reviews* **7,** *307* (*1949*); **8,** *48* (*1950*)).

Experimental observations of H. McIlwain, D. A. Stanley, and D. E. Hughes (*Biochem. J.* **44,** *153* (*1949*)) have demonstrated synthesis of DPN from niacin by the bacterium, *Lactobacillus arabinosus*. The process of synthesis may be followed through the increased glycolytic activity of washed bacterial cells after growth on a medium deficient in niacin. Since the organism does not destroy the coenzyme rapidly, it offers a tool suitable for the investigation of the bacterial synthesis of DPN.

In a recent report, experiments are described in which the effectiveness of niacin and certain of its derivatives on the synthesis of DPN by *L. arabinosus* was examined (D. E. Hughes and D. H. Williamson, *Biochem. J.* **51,** *330* (*1952*)). The organisms were grown on a medium deficient in niacin for a period of thirty to forty-four hours. These cells were centrifuged, washed, and the rate of glycolysis determined in a manometric system. Addition of niacin to the system increased the rate of glycolysis two to three times. After determination of the rate of glycolysis the cells were removed, washed, and the concentration of DPN determined. The quantity of niacin or derivatives of niacin utilized in coenzyme synthesis was also determined.

Results demonstrated that there was a lag period before niacin in the medium manifested an effect on glycolysis. The length of this lag period varied from ten to fifty minutes under the conditions of these experiments and was conditioned by the quantity of niacin added. The lag period apparently represents the time required for the bacterial cells to synthesize a quantity of coenzyme sufficient to meet the needs of glycolysis. It was determined that the quantity of niacin necessary to saturate this system was 1.0 to 2.0 micromoles per gram

dry weight of organisms. These results were confirmed by determination of the DPN content of organisms incubated in a medium containing niacin. Synthesis of coenzyme began promptly with addition of niacin and proceeded at a maximum rate with concentrations of the vitamin comparable to those which permitted saturation of the glycolytic system. All of the pyridine nucleotide synthesized was found in the cells. None was present in the cell-free medium. The growth phase of the bacteria was an important factor determining rate of synthesis. Maximum rates occurred in organisms after sixteen to twenty hours incubation in the niacin-deficient growth medium. Periods of incubation either shorter or longer than this were associated with lesser rates of synthesis.

Various derivatives of niacin were added to the glycolytic system to determine their availability as precursors of DPN. With young cells grown in the deficient medium for a period of from nineteen to twenty-two hours, niacinamide, niacinamide riboside (dihydroniacinamide ribofuranoside) and niacinamide ribotide (niacinamide ribose phosphate) were as effective as niacin as determined by stimulation of glycolysis or increase in the rate of coenzyme synthesis. With older cells of thirty to forty-eight hours growth, niacinamide and its derivatives stimulated glycolysis more rapidly than niacin, although final glycolytic rates were the same. Determination of the pyridine nucleotide content of these cells failed to demonstrate differences in rates of synthesis. Other derivatives added to the system failed to influence coenzyme synthesis. Included were niacinamide arabinoside, niacinamide glucoside, and niacinamide galactoside.

Evidence is presented indicating that preformed DPN is readily taken up by the cells. An energy source is required for this absorption since it did not occur in the absence of glucose. The maximum concentrations of DPN achieved by deficient cells incubated in media containing niacin, its amide, or preformed DPN were the same, 10 to 12 micromoles per gram dry weight of organisms. This concentration is identical with that found in other cells grown on a complete medium which was not deficient in niacin. When this level is reached, cells either fail to absorb or synthesize further coenzyme, or actually destroy it.

Niacin was taken up from the medium at approximately the same rate as that of coenzyme synthesis in the cells and did not occur in the absence of glucose. Small quantities of free niacin were sometimes found in cells. When the maximum concentration of DPN was reached in the cells, there was no further disappearance of niacin from the medium. No other derivatives or intermediates were found during usual periods of one to two hours of incubation. However, with incubation for longer periods, up to four hours, small quantities of an N_1-substituted derivative of niacinamide accumulated in the medium. Further identification of this compound was not achieved.

Niacinamide added to the washed glycolyzing cells disappeared from the medium rapidly and was not fully accounted for by the DPN synthesized. Further investigation demonstrated that niacinamide was being deamidated since niacin and ammonia could be recovered from the medium. This reaction did not require glucose. Deamidation of niacinamide proceeded at a rate two hundred times as fast as the synthesis of DPN from niacinamide and was shown to be independent of the pyridine nucleotide content of the bacterial cells.

In additional experiments other constituents of a semisynthetic bacterial medium were tested for their effects on DPN synthesis from niacin by *L. arabinosus*. Of the substances tested, only glucose was shown to be necessary and, as indicated above, synthesis occurred only when glycolysis was going on. It occurred under aerobic and anaerobic conditions and in a wide range of hydrogen ion concentration. It could be stated that synthesis of DPN by this or-

ganism was not limited by precursors of parts of the molecule other than niacin.

These observations indicate that niacin is more readily available for synthesis of DPN by *L. arabinosus* than is niacinamide and that niacinamide functions as an effective precursor only because the organism rapidly releases niacin through deamidation. This is in contrast to the finding in most other bacterial species in which niacinamide seems to function more readily as a precursor (B. C. J. G. Knight, *Vitamins and Hormones* **3,** *105* (*1945*)). However, certain strains of *Leuconostoc mesenteroides* require niacin for growth and niacinamide is ineffective in replacing niacin (*Nutrition Reviews* **7,** *133* (*1949*)). It should be recalled that DPN accumulates in human erythrocytes incubated in a medium containing niacin, but not in one which includes niacinamide (P. Handler and H. I. Kohn, *J. Biol. Chem.* **150,** *447* (*1943*)). It may be concluded that pathways of synthesis of DPN may vary in different species. The possibility remains that in certain bacterial species and perhaps in human erythrocytes the free acid is the primary precursor and amidation of niacin is associated with a later phase in the synthesis of DPN.

EFFECT OF FOLACIN ON BLOOD PRESSURE

A number of observations have indicated that folacin and ascorbic acid are interrelated in certain of their metabolic functions. One series of reports has demonstrated that the urinary excretion of phenolic degradation products of tyrosine by scorbutic infants and guinea pigs is decreased or abolished when folacin is administered (*Nutrition Reviews* **8,** *260* (*1950*)). Another relates to the occurrence of megaloblastic anemia in monkeys on diets deficient in folacin and ascorbic acid, with correction of the hematologic defect when either of the two vitamins is supplied (*Ibid.* **9,** *52* (*1951*)).

R. E. Lee and E. A. Holze (*Proc. Soc. Exp. Biol. Med.* **76,** *325* (*1951*)), in an evaluation of the effect of ascorbic acid on hemodynamic mechanisms, found that guinea pigs deficient in ascorbic acid respond poorly to hemorrhagic shock and that this apparently is due to failure to elaborate the vasoexcitor substance (VEM) in the kidney. These experiments have been extended in the attempt to determine if folacin might again have a function parallel to or related to that of ascorbic acid in regulation of blood pressure (Lee, S. Tanaka, and Holze, *Circulation* **5,** *903* (*1952*)).

Young growing rats were the experimental animals utilized in these studies. They were placed at an age of 21 days on a diet of well defined constituents which was free of folacin. They were maintained on this diet for a period of six weeks. One group of 10 animals received no dietary supplement of any kind for the entire experimental period. Another group of 17 rats was given folacin in quantities of 1.0 mg. per 100 g. of body weight three times a week. This was administered in aqueous solution by intraperitoneal injection. A third group of 22 animals served as a control group and was maintained on a diet of laboratory pellets without additional folacin. Animals of the same sex and litter were distributed as evenly as possible among the three groups. After six weeks the mean blood pressure in the carotid artery of each animal was determined under general anesthesia, the animals were killed, and specimens of various tissues were taken for microscopic study and determination of ascorbic acid.

The blood pressures of animals on the stock diet and on the folacin-deficient diet were essentially the same. There was a significant increase in the mean carotid blood pressure of the rats which were given the deficient diet but were also provided supplements of folacin by injection. The mean blood pressure in this group was 172 mm. of

mercury. This compared with pressures of 143 and 145 mm. of mercury in the groups on the folacin-deficient diet and the stock diet respectively. The difference is one which is of significance by statistical analysis.

Concentrations of ascorbic acid in tissues of the kidney, liver and adrenals of animals on the deficient diet with and without supplements of folacin were comparable and did not differ by significant degrees. Comparing tissue concentrations of animals on the deficient diet with those on the stock diet, it was found that the kidney content of ascorbic acid was lower and the liver content higher in the group on the deficient diet. These differences are not stated to be significant, but the data recorded would indicate that the higher concentration of ascorbic acid in the livers of the group of animals on the folacin-deficient diet is of significance. No explanation is offered for these differences. The values recorded for adrenal concentrations of the vitamin, about 300 mg. per 100 g., are low as compared with similar data in the literature for rats in a resting phase. Low values may probably have resulted from the stress of anesthesia and direct determination of arterial blood pressure prior to sacrifice. It is of some interest to note that the deficient diet undoubtedly contains a source of vitamin C. The rat, of course, is a species which does not require a dietary source of vitamin C.

The investigators obtained no information which would indicate that ascorbic acid and folacin are interrelated in terms of metabolic functions which pertain to blood pressure-regulating mechanisms. The incidental finding that provision of folacin by injection to rats on a diet deficient in this vitamin was associated with increase in blood pressure is of interest, however, and has not been previously observed. In seeking an explanation of this phenomenon the authors found scarring of the cortex of the kidney in 15 of the 17 rats which received folacin, while these lesions were not present in the control animals. The scarred areas were characterized by increase in fibrous tissue and damage to cells of the proximal and distal convoluted tubules.

Other investigators have previously described damage to the tubules of the kidney in animals receiving folacin by parenteral injection. One such report was that of B. K. Harned, R. W. Cunningham, H. D. Smith, and M. C. Clark (*Ann. N. Y. Acad. Sci.* **48,** *289 (1946)*). The lesion was observed in rabbits which received 50 mg. folacin per kilogram per day for ten weeks by an intraperitoneal route and in rats given 75 mg. intraperitoneally per kilogram per day for nine weeks. A somewhat similar lesion was observed by J. R. Dawson, C. W. Woodruff, and W. J. Darby (*Proc. Soc. Exp. Biol. Med.* **73,** *646 (1950)*) in guinea pigs given parenteral folacin in quantities of 1.0 mg. daily for twenty-one days. In these experiments the kidney tubules were dilated and there were casts within the tubules. It was stated that the casts were probably composed of folacin. The lesions were observed in animals on scorbutic diets and it was found that they did not occur in guinea pigs fed a stock diet containing cabbage.

In the experiments of Lee and his associates (*loc. cit.*) the quantity of folacin administered was considerably less than in the earlier experiments in which renal damage was observed. No tubular casts were found. It would have been of added interest to determine whether or not tubular damage occurred in rats on stock diets given folacin parenterally. The indication in the observations of Dawson *et al.* (*loc. cit.*) is that factors in the stock diet of guinea pigs protected against the effect.

That the blood pressure was uniformly elevated in rats provided folacin by injection may relate directly to the associated kidney damage as in renal lesions of other origins. However, it is suggested that metabolic mechanisms of another type might conceivably be involved. Folacin has been shown to have an inhibitory effect on certain of the oxidase enzymes. This might account

for interference with enzyme systems which are involved in inactivation of endogenous hypertensive factors. Since folacin has also been demonstrated to accelerate processes of transmethylation, an excess of the vitamin might lead to a relative insufficiency of methyl donors, and then to hypertension as has been observed in rats on choline-deficient diets. Neither of these possible modes of action has been demonstrated as the cause of hypertension in the rats in the experiments described above.

The hypertension observed in these animals does not stand as an isolated finding. Harned and his associates (*loc. cit.*) found a transient rise in blood pressure following a single intravenous injection of folacin in cats, dogs, and rabbits. It is entirely likely that the vitamin may have an important direct effect on vasoregulatory mechanisms. It may be hoped that these observations will be extended in a search for more adequate explanations of this effect of folacin.

PROTEIN AND JOINT DISEASE IN MICE

The metabolic aspects of joint disease have recently assumed new importance (*Nutrition Reviews* **8,** *173 (1950)*). The development of a strain of C_{57} black mice, in which spontaneous articular changes resembling the nonrheumatoid degenerative arthritis of humans occur, has afforded an opportunity for investigation of certain nutritional aspects of this disease (M. Silberberg and R. Silberberg, *Arch. Path.* **48,** *331 (1949)*). The investigators have reported modification of the course of these joint lesions by the use of hormones and acceleration of skeletal aging and increased incidence of arthritis when 29 per cent instead of 5 per cent fat was used in the diet from the time of weaning (*Ibid.* **50,** *828 (1950)*). By contrast, mice fed a 53 per cent casein diet from weaning showed accelerated aging processes in the joints.

In a more recent study these workers (M. Silberberg and R. Silberberg, *J. Gerontol.* **7,** *24 (1952)*) have reported the effects of protein-enriched diets fed to mice of this strain throughout life. Groups of animals were fed casein-enriched 53 per cent protein and fishmeal-enriched 46 per cent protein diets while the control group received a stock diet of 26 per cent protein. Little difference was noted in the growth curves of these groups, although the average weight of the control mice exceeded the special diet animals for the first eighteen months of life. Articular changes occurring in the various groups were followed by studies of animals killed at 9 to 12, 13 to 18, and 19 to 24 months of age and the findings were classified as "no change," slight or advanced "age change," and "osteoarthritis."

Delay in articular aging and acceleration of skeletal development was observed in the animals on the protein-enriched diet as compared with the controls. At the earliest age (9 to 12 months) there was no change in joint appearance in 43 and 40 per cent of the casein- and fishmeal-fed animals respectively, while only 22 per cent of the controls were without change. Similar relationships were found in the 13 to 18 month category, with osteoarthritis being present in 32 per cent of the controls compared to 21 and 19 per cent respectively of the casein- and fishmeal-fed mice. At the age of 19 to 24 months 55 per cent of the controls had developed osteoarthritic joints compared to 45 per cent of the casein-fed group. Too few of the fishmeal-fed mice were studied to allow comparison. Figures derived from the combined age categories of the three diet groups reveal that the control mice had less animals with normal joints (13 per cent) and more animals with osteoarthritis (32 per cent) than either of the other groups. Twenty-one per cent of casein-fed animals

and 30 per cent of fishmeal-fed animals were without joint lesions. Osteoarthritis was present in 28 per cent of the former and 14 per cent of the latter. The high incidence of osteoarthritis in the former (casein-fed) animals may have been due to an older average age in these mice (19.8 months) as compared with the control and fishmeal groups (14.2 and 15.7 months). For the classification "age changes," little difference between the groups is noted except that the control mice exhibited slightly more "slight age change" and less "advanced age change" than the experimental mice.

From the data presented it appears that although not free of articular disease, the high protein-fed animals had a reduced incidence of arthritis and a higher percentage of normal joints regardless of the age of the animals. The mechanism of the protection afforded by the use of a high protein diet in mice spontaneously developing articular changes is still obscure, but the authors point out that the use of the diet from birth suggests that the type of diet eaten at an early age may influence joint disease which is associated with aging. In view of their previous studies with hormones (*see above*) it would be of interest to know what changes, if any, were produced in the endocrine glands of the animals fed high protein diets. The extension of this type of investigation may yet prove fruitful in understanding the metabolic changes affecting articular lesions of the degenerative type.

THE TURNOVER RATE OF FIBRINOGEN

The pioneer work of S. C. Madden and G. H. Whipple (*Physiol. Rev.* **20,** *194 (1940)*; *Nutrition Reviews* **1,** *200 (1943)*) demonstrated that blood proteins, like other organic constituents of the body, are in a constant state of "dynamic equilibrium." They and other authors used the plasmaphoresis technic as a tool for study of the biologic value of dietary proteins when synthesis of plasma proteins is accelerated through massive depletion (*Ibid.* **2,** *262, 322 (1944)*).

Among the various blood proteins, it was early shown (W. J. Meek, *Am. J. Physiol.* **30,** *161 (1912)*; D. P. Foster and G. H. Whipple, *Ibid.* **58,** *407 (1921–1922)*) that fibrinogen falls particularly rapidly after hepatectomy or severe liver damage; in some cases, zero levels were observed in the dog within thirty hours after the operation or toxic trauma. From these and similar determinations, it can be calculated that the half-life of fibrinogen in hepatectomized animals is sixteen to twenty-four hours. That this type of experimental data can be directly translated into a quantitative rate of catabolism and of synthesis of fibrinogen is, however, open to question when one considers the profoundly unphysiologic state of the hepatectomized animal. A recent report of R. E. Madden and R. G. Gould (*J. Biol. Chem.* **196,** *641 (1952)*), describing the direct determination, through the use of isotopic technics, of the turnover rate of fibrinogen in the normal, intact dog, eliminates this limitation.

The experimental procedure used by Madden and Gould was simple: dogs were fed synthetic methionine labeled with radioactive sulfur (S^{35}) in some experiments and S^{35}-labeled yeast in others. The S^{35} content of fibrinogen was determined by isolation and assay of fibrin. The specific activity of fibrin was followed while labeled methionine was fed in a standard daily dose until a constant value was obtained. It was also followed subsequent to a period of labeled-methionine or -yeast feeding, during the decreasing activity phase. Both types of determinations were found to be in substantial agreement. The "uptake" experiments yielded half-turnover times (cor-

responding to 50 per cent replacement) varying from 4.1 to 4.6 days, while the "die-away" experiments gave values ranging from 3.9 to 4.2 days. The half-turnover time for fibrinogen can reasonably be accepted therefore to be 4.2 days in the dog. The relative specific activity of the total serum protein fraction was followed simultaneously by a similar method; a mean of 11.4 days for 50 per cent turnover was found in experimental conditions.

From the half-turnover time found for fibrinogen, it can be calculated that the turnover time (defined as the time necessary for the synthesis of an amount of fibrinogen equal to the total amount present in the animal at any given time) is 6.0 days. That this value may be somewhat too high is possible if some degree of reutilization of the labeled sulfur obtained by breakdown of the labeled methionine took place. In view of the fact that inert sulfur-containing amino acids were administered with the tracers, it seems likely, however, that the overvaluation will be large. While the value of 6 days corresponds to a turnover rate faster than that of other blood proteins, it indicates that in normal, intact animals, the rate of disappearance of fibrinogen is very much less than that found previously in hepatectomized dogs. No explanation is offered for this discrepancy, but the authors suggest that, in normal animals, one important type of utilization of fibrinogen is the repair by fibrin of continually developing minute leaks in the vascular and lymphatic systems.

Preliminary determinations of fibrinogen turnover rate in 3 human patients were also conducted by Madden and Gould. Values of the same order of magnitude were found to prevail for men as had been found in dogs. There again, the rates found are probably overvaluation; for the 3 individuals studied the average turnover time was 8.1 days.

These experiments give one more illustration of the irreplaceable value of isotopic methods for the determination of reaction rates *in vivo*. They also demonstrate how inaccurate it may be to extrapolate values obtained from results on animals subjected to extensive surgical or toxic trauma, to those characteristic of normal animals or humans.

NOTES

On Wednesday afternoon, September 10, 1952, Mr. Ole Salthe, Executive Secretary of The Nutrition Foundation, died. Mr. Salthe always had the best interests of *NUTRITION REVIEWS* at the top of his list of activities and responsibilities. He was continually thinking of ways to improve the journal and increase its circulation and acceptance, particularly by the medical profession. *NUTRITION REVIEWS* has lost its best friend and strongest supporter—unselfish, sincere, modest, discerning, candid—we shall miss him greatly.

Letter to the Editor

Dear Sir:

In the July 1952 issue of *Nutrition Reviews* (**10,** *211* (*1952*)) appears an abstract entitled "Aureomycin, Dietary Proteins and the Growth of Mice."

The first sentences in this review state, "Aureomycin was the *first* antibiotic for which growth-promoting activity was suggested. The chick was used as the experimental animal (E. L. R. Stokstad and co-workers, *J. Biol. Chem.* **180,** *647* (*1949*); Stokstad and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **73,** *523* (*1950*))."

Just to keep the record straight, and in the interest of historical accuracy, in *1946* P. R. Moore, A. Evenson, T. D. Luckey, E. McCoy, C. A. Elvehjem, and E. B. Hart published in the *J. Biol. Chem.* (**165,** *437* (*1946*)) an article entitled, "Use of Sulfasuxidine, Streptothricin, and Streptomycin in Nutritional Studies with the Chick." Most writers on antibiotics in nutrition, giving a historical review, refer to this article as the initial one in the field. Quoting from the summary, "Sulfasuxidine and streptomycin singly or in combination lead to increased growth responses in chicks receiving our basal diet supplemented with adequate amounts of folic acid."

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Determination of Bound Ascorbic Acid

The existence of bound ascorbic acid in plant tissues was described by E. J. Reedman and E. W. McHenry (*Biochem. J.* **32,** *85* (*1938*)). Various investigators followed this finding by attempts to determine bound ascorbic acid in animal tissues, but the lack of a satisfactory method prevented agreement on analytic results.

W. N. Sumerwell and R. R. Sealock (*J. Biol. Chem.* **196,** *753* (*1952*)) have devised a procedure which seems to give reproducible and reliable values for liver tissue. The sample is repeatedly extracted with 95 per cent ethyl alcohol in an atmosphere of carbon dioxide. After centrifugation, the free ascorbic acid is determined by the 2,4-dinitrophenylhydrazine method (J. H. Roe and C. A. Kuether, *Ibid.* **147,** *399* (*1943*)). Bound ascorbic acid is then separated from the protein through a thirty-minute hydrolysis in a 5 per cent solution of metaphosphoric acid and also determined by the Roe-Kuether method. Repeated determinations indicate that pork liver contains about 18 per cent of its ascorbic acid in the bound form.

In view of the appreciable amount of ascorbic acid found in the bound form, the authors question the accuracy of hitherto accepted ascorbic acid contents of foods. They also suggest that the bound ascorbic acid may represent the enzymatically active form of the vitamin.

CLINICAL NUTRITION BRIEFS

"The data suggest that with the possible exception of arginine and threonine there are no sustained decreases in plasma concentration of the 'essential' amino acids during the first week following major surgical operations in previously well nourished patients." (*Nutrition Reviews* **10,** *195* (*1952*), Effect of Surgery on Plasma Levels of Essential Amino Acids.)

"During the last decade there has been increasing interest in the role played by the intestinal flora in mammalian and, more recently, in human nutrition. Intestinal synthesis of vitamin K is of great importance in maintaining nutriture with regard to this vitamin. The excretion of large amounts of the B-complex vitamins in the feces of experimental animals and man, sometimes quantitatively exceeding the intake, would suggest that these supplies might also be available to the host organism." (*Nutrition Reviews* **10,** *198* (*1952*), The Intestinal Flora of Infants.)

"These investigations not only confirm previous studies of this type of clinical problem, but also suggest that 'synthetic' diets have no advantage over natural types of foods and that a certain degree of accommodation or recovery occurs with the passage of time in patients with decreased intestinal surface area." (*Nutrition Reviews* **10,** *202* (*1952*), Nutrient Absorption Following Intestinal Resection.)

"On the basis of present knowledge, the question of whether or not malnutrition influences the incidence and extent of dental fluorosis cannot be answered with any degree of certainty." (*Nutrition Reviews* **10,** *227* (*1952*), Relation of Malnutrition to Fluoride Toxicity.)

"While the list of substances found *not* to be Castle's intrinsic factor continues to grow, the over-all physiologic role of intrinsic factor in aiding the absorption of vitamin B_{12} from the gastrointestinal tract seems firmly established by the clinical evidence." (*Nutrition Reviews* **10,** *229* (*1952*), Nature and Mode of Action of the Intrinsic Factor.)

"These investigations approach the problem of lipotropic agents in human liver disease in different ways. The tentative conclusions which they reach, however, are not widely separated and the value of these agents would appear to be limited to the initial phases of therapy in patients with fatty infiltration, with little benefit to be derived thereafter when patients are able to ingest adequate diets." (*Nutrition Reviews* **10,** *259* (*1952*), Lipotropic Supplements in Fatty Cirrhosis.)

"The edema is almost invariably associated with hypoalbuminemia. Hyperglobulinemia is also frequently found, and the total serum proteins may approach normal values. Fatty infiltration of the liver is an extremely common postmortem finding. In some areas fibrosis without fatty change has been described. This may be due to differences in the carbohydrate supplements given to the very young infants." (*Nutrition Reviews* **10,** *261* (*1952*), Kwashiorkor in Africa.)

"The data are consistent with the concept that in severe portal cirrhosis there is probably a marked interference with albumin synthesis." (*Nutrition Reviews* **10,** *268* (*1952*), Iodine-Tagged Albumin in Cirrhotic Patients.)